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Synthesis of potential prodrug systems for reductive activation. Prodrugs for anti-angiogenic isoflavones and VEGF receptor tyrosine kinase inhibitory oxindoles

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ABSTRACT

A number of potential prodrug systems for reductive activation have been investigated. The prodrug systems chosen for the study were the 2-nitrophenylacetyl, 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoyl and 4-nitrobenzyl groups, readily attached to acidic OH or NH groups in drug molecules, and released upon bioreductive activation. The drug molecules studied were the naturally occurring isoflavone biochanin A, an inhibitor of VEGF-induced angiogenesis, and the pyrrolylmethylidenyl oxindole SU5416 (semaxanib) and its 6-hydroxy derivative, inhibitors of VEGF receptor tyrosine kinase. Following coupling the prodrug system to the drug, the compounds were evaluated chemically and biologically. Under chemical reducing conditions, the 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid based prodrugs appear to fragment the most efficiently, followed by the 2-nitrophenylacetate esters with the 4-nitrobenzyl ethers being the least efficient. The potentially pro-anti-angiogenic compounds were also assayed for their ability to block VEGF-induced angiogenesis in HUVECS in comparison to the free agents. Control compounds that cannot be activated under bioreductive conditions are less potent than the free drug, whereas many of the potential prodrugs not only exhibit a dose response, but appear at least equipotent with the free drug.

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1. Introduction

The design of prodrug systems for use in cancer therapy is a topic of current interest.[1] Such prodrugs have the potential to improve tumour selectivity of chemotherapeutic agents and hence reduce unwanted toxic side effects. One prodrug strategy that has been widely investigated uses bioreduction of readily reducible compounds such as quinones, nitro aromatics or *N*-oxides, to release the active drug from its prodrug.[2–6] This strategy takes two forms. Firstly, one can rely on the combination of hypoxia and the upregulation of reductase enzymes such as NQO1 (2-electron reductase) and cytochrome c reductase (1-electron) to effect a tumour selective reduction that releases the active species. In the second approach, an exogenous activating enzyme is delivered to tumour cells using antibody or gene therapy (ADEPT or GDEPT), with the most common exogenous reductase being a bacterial nitroreductase.

Some examples of bioreductive prodrug strategies are shown for nitroarenes and quinones in Scheme 1. For illustrative purposes we only show reduction of the nitro groups to hydroxylamino, followed by lone-pair initiated fragmentation. The product of further reduction, the aniline, would behave similarly. Likewise we only show reduction of quinones to hydroquinones by a 2-electron process, although it is recognized that for both nitroarenes and quinones, bioreductive activation can also proceed by 1-electron pathways involving radical anion intermediates. In the first two examples the reduction process results in release of the active drug **DH**, after protonation of the leaving group $\mathbf{D}^-$ in water, with the formation of a relatively innocuous byproduct. Thus reduction of the 2-nitrophenylacetyl derivative **1** (R=H or Me) would lead to the corresponding hydroxylamine **2** followed by spontaneous cyclization to the *N*-hydroxyoxindole **3** with concomitant release of **DH**.[7] Likewise, in the second example, reduction of the quinone **4** to the corresponding hydroquinone **5** results in rapid lactonization to give **6** with expulsion of the leaving group;[8,9] the method has been used as

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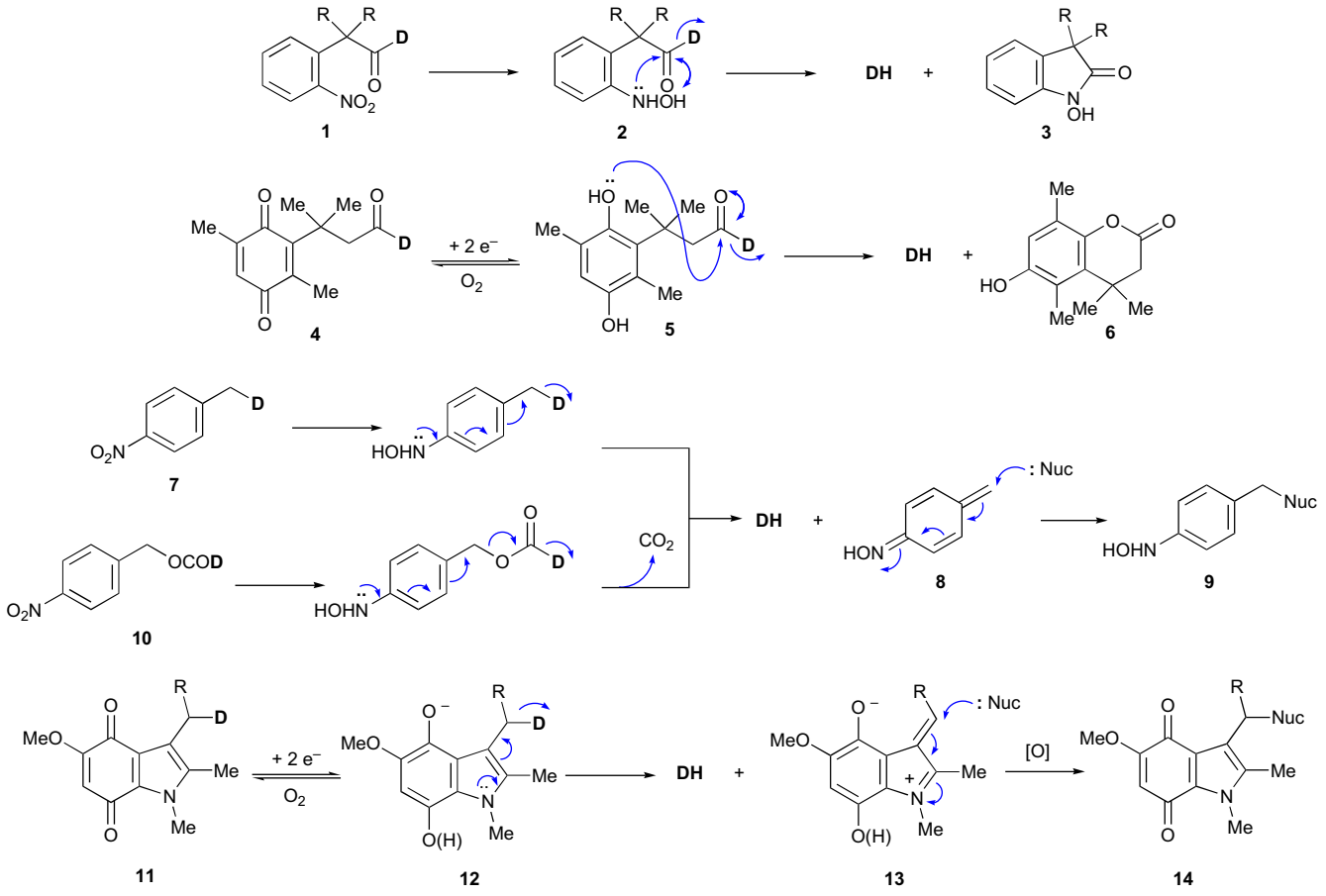


Scheme 1. Proposed mechanisms of bioreductively initiated release of drugs **DH** from nitroarene and quinone prodrugs.

a bioreductive drug delivery system for melphalan.[10,11] In the other examples shown in Scheme 1 release of the drug **DH** results in the generation of an electrophilic species, which could also exert its own cytotoxic effect. The most common systems of this type involve the nitrobenzyl group as shown for the 4-nitrobenzyl compound **7**, bioreduction of which would lead to the release of the drug **DH** simultaneous with formation of a reactive electrophile **8**. Interception of **8** by a biological nucleophile (DNA or protein) then leads to irreversible modification of the biomolecule and hence toxicity. The corresponding nitrobenzyloxycarbonyl compound **10** behaves similarly. The 4-nitrobenzyl derivatives **7** have found use as prodrugs for phosphoramide mustards,[12,13] whereas the nitrobenzyloxycarbonyl compounds **10** have been widely applied in GDEPT,[6] as prodrugs for, for example, minor groove alkylating agents,[14] doxorubicin[15] and cytotoxic acridines.[16] Although all the nitro compounds shown in Scheme 1 are nitrobenzene derivatives, bioreductive prodrugs based on nitro-imidazoles, -furans and -thiophenes have also been developed.[17–23] Our own contributions in this area have involved the indolequinone **11** prodrug system, reduction of which gives a hydroquinone **12** capable of releasing drug **DH** whilst simultaneously generating the iminium electrophile **13**, reaction of which with biological nucleophiles gives, after reoxidation, the adduct **14**.[24–28] We have studied this quinone system using various model drugs, whilst others have used PARP inhibitors[29] and camptothecin.[22]

In continuation of our interest in bioreductively activated drugs,[24–28,30] and in anti-angiogenic agents,[31] we initiated a study of potential prodrugs for anti-angiogenic isoflavones and VEGF receptor tyrosine kinase inhibitory oxindoles, and we now report the full details of this work.[32]

2. Results and discussion

The development of the microvasculature is a critical aspect of tumour growth as recognized by Folkman some 35 years ago.[33,34] This process of angiogenesis is a prerequisite for tumours to grow beyond the minimum volume, and although it is stimulated by a number of factors, there is considerable evidence that vascular endothelial growth factor (VEGF) is a major contributor to solid tumour growth by the promotion of both angiogenesis and vascular permeability.[35,36] Hence the inhibition of VEGF expression, induction or function represents an attractive target for the development of novel therapeutic agents. To date a number of small molecule inhibitors of VEGF receptor tyrosine kinases have been developed and are in clinical trial,[37] with Avastin® (bevacizumab), a monoclonal antibody against VEGF and Sutent® (sunitinib) already receiving FDA approval.

The compounds chosen for study were the isoflavone biochanin A **15**, the oxindole SU5416 **16** and its 6-hydroxy derivative **17** (Fig. 1). Biochanin A **15**, and other soya-derived isoflavones such as genistein, are known to block the effects of VEGF,[31,38,39] and are readily functionalized through the 7-hydroxy group. The oxindole SU5416 **16**, also known as semaxanib, is a potent inhibitor of the VEGF receptor tyrosine kinase Flk-1/KDR, and a proven anti-angiogenic agent,[40–43] although its clinical development has now been discontinued. A related oxindole (SU11248) is now marketed as Sutent® (sunitinib). Oxindoles are readily functionalized through their NH group, although we have also prepared the 6-hydroxy derivative **17** to provide an alternative point of attachment of the prodrug moiety.

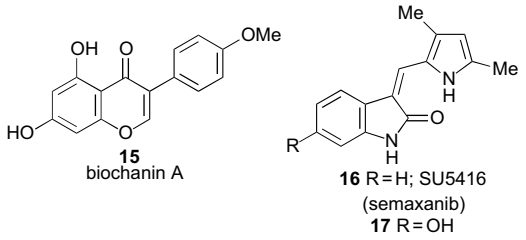


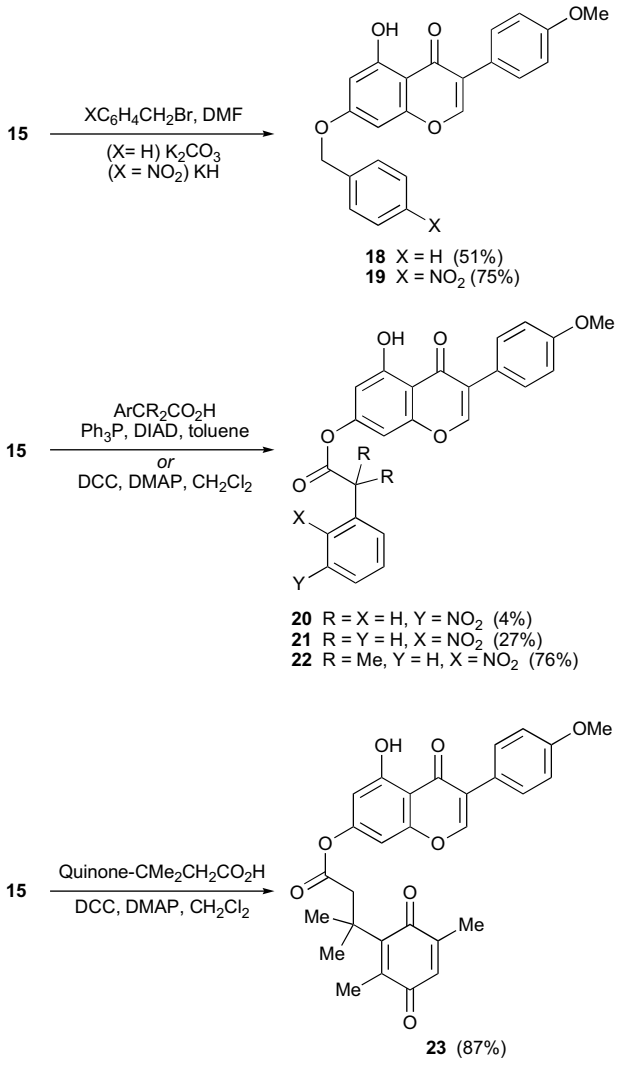
Figure 1. Structures of anti-angiogenic agents studied.

Initially, commercially available biochanin A **15** was converted into the benzyl and 4-nitrobenzyl ethers **18** and **19** by alkylation reactions. As expected, the two hydroxyl groups in biochanin A exhibit different reactivity due to the involvement of the 5-OH in intramolecular hydrogen bonding, and therefore alkylation occurred predominantly at the 7-OH group, as confirmed by NOE NMR spectroscopy. Nitro-substituted phenylacetic acids were readily attached to biochanin A using Mitsunobu or carbodiimide coupling protocols to give compounds **20**–**22**, the dimethyl substituted precursor to **22** being prepared by a literature method.[7] Finally the quinone derivative **23** was obtained by coupling to 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid[44] (Scheme 2). The benzyl and 3-nitrophenylacetyl derivatives **18** and **20** are prepared as controls since they cannot participate in the reductively activated fragmentations shown in Scheme 1. This is particularly important in the case of the nitrophenylacetate esters since they could also release the free drug by a hydrolytic rather than a reductive process.

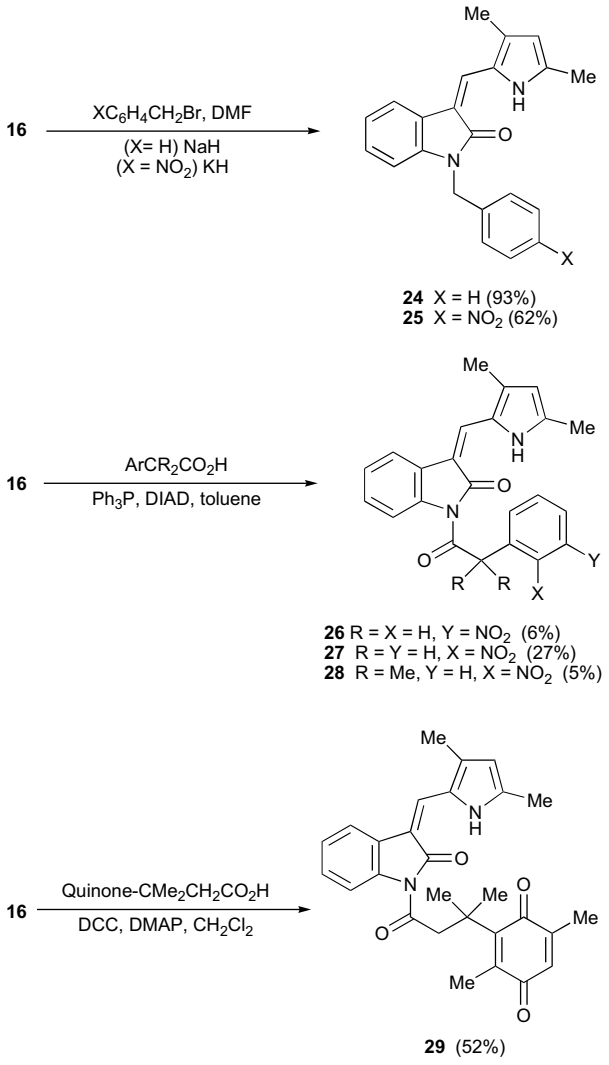
An analogous set of derivatives **24**–**29** was prepared from SU5416 **16**, obtained from condensation of oxindole with 3,5-dimethylpyrrole-2-caboxaldehyde,[45] using similar protocols, as outlined in Scheme 3. No attempt was made to optimize the rather poor yields observed in some of these coupling reactions.

Finally, the 6-hydroxy derivative **17** of SU5416 was investigated. The starting material, 6-hydroxyoxindole,[46] was converted into the corresponding benzyl ether, reaction of which with 3,5-dimethyl pyrrole-2-aldehyde gave the SU5416 derivative **30**. Alternatively, reaction of 6-hydroxyoxindole with the pyrrole aldehyde gave 6-hydroxy SU5416 **17** in good yield. Subsequent reactions of **17** with 4-nitrobenzyl bromide, 2-nitrophenylacetic acid or the aforementioned benzoquinonylbutanoic acid gave the novel derivatives **31**–**33** (Scheme 4).

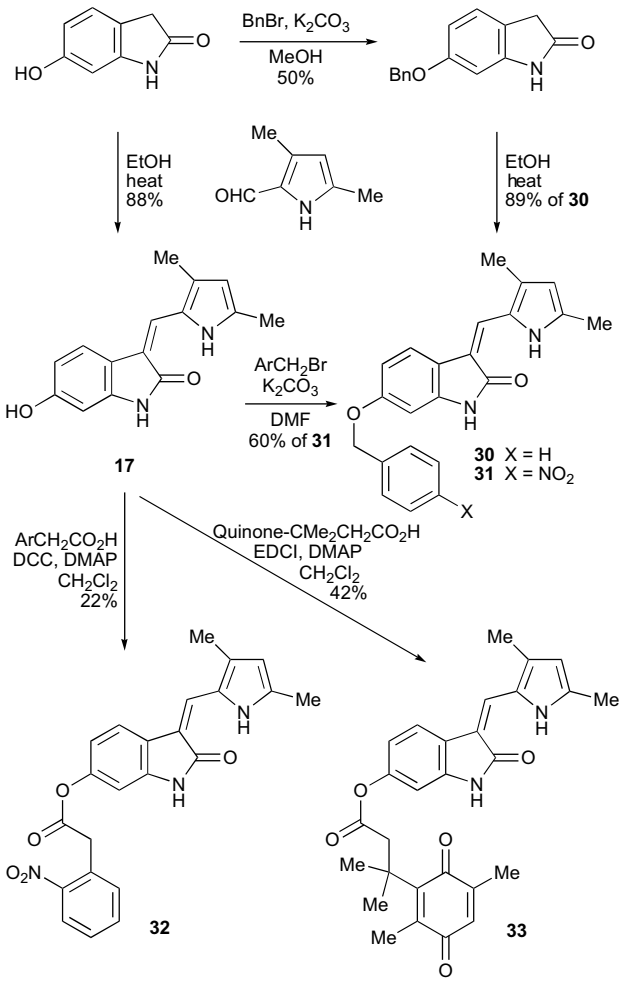
In order to assess the ability of the various prodrug systems to release their phenolate or oxindole leaving group upon reductive activation, selected compounds were subjected to a simple chemical reduction. Thus 7-(4-nitrobenzyloxy)biochanin A **19** was treated with indium powder in ethanolic ammonium chloride solution, an efficient method for the reduction of nitroarenes to



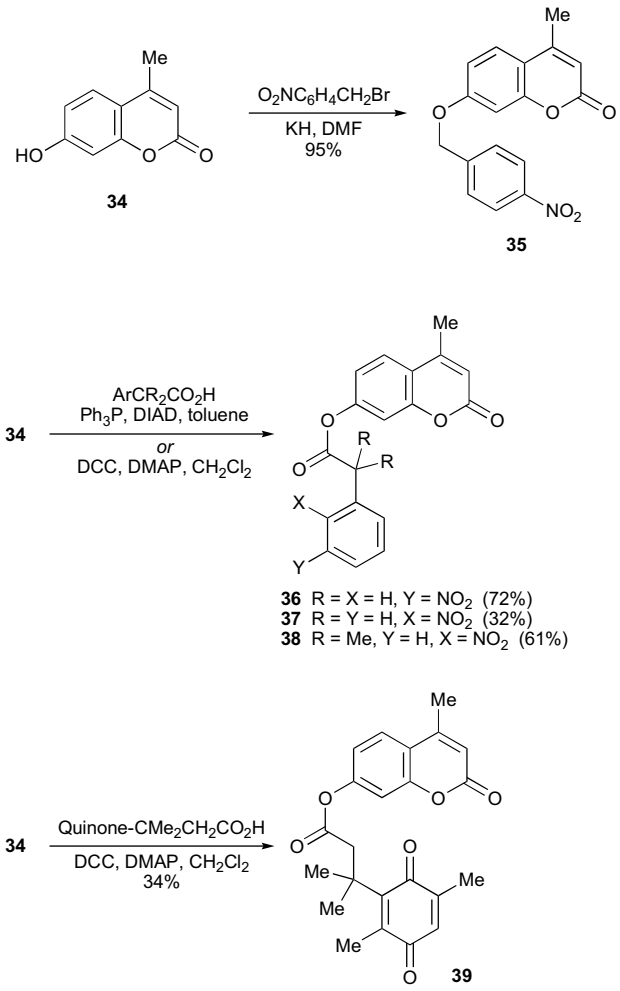
Scheme 2.



Scheme 3.



Scheme 4. Yields in scheme clarified.



Scheme 5.

anilines[47] and the reaction monitored by NMR spectroscopy. The signal for the benzylic CH_2 group at ca. δ 5.20 disappeared after about 40 min, concomitant with the appearance of signals due to free biochanin A. Likewise reduction of the 2-nitrophenylacetyl derivative **21** also triggers the release of biochanin A. In the oxindole series, reduction of the 4-nitrobenzyl compound **25** proceeded cleanly to give the corresponding 4-aminobenzyl derivative that did not fragment further. In contrast, indium reduction of the 2-nitrophenylacetyl derivative **27** did result in release of free SU5416 **16**.

For a more complete study of the ability of the prodrug systems to fragment upon reductive activation, a model system was also investigated. Thus the strongly fluorescent compound, 7-hydroxy-4-methylcoumarin **34**, was functionalized as described previously for biochanin A **15** to give the coumarin derivatives **35**–**39** (Scheme 5). These compounds in which the coumarin 7-OH is blocked are essentially non-fluorescent and hence can act as pro-fluorescent indicators for efficient reductive activation leading to release of the fluorophore itself. A similar approach has been used with 7-aminocoumarin derivatives.[48]

The pro-fluorescent compounds (10 μmol) were dissolved in a chloroform–ethanol–water mixture in a cuvette and treated with an excess of sodium dithionite as reducing agent. The progress of the reaction was monitored by following the fluorescence at 445 nm (excitation wavelength 360 nm) in a fluorescence spectrometer pre-calibrated so as to reach 70% fluorescence for 10 μmol of free coumarin. It was immediately apparent that under these conditions release of coumarin from the 4-nitrobenzyl ether **35** was very slow, whereas the 2-nitrophenylacetic acid derivative **37**, and the benzoquinone **39** underwent fragmentation with release of coumarin as shown in Figure 2. In order to check that this was due to reductive activation rather than simple hydrolysis of the phenylacetate esters, the 3-nitro compound **36**, in which there is no possibility for mechanism based reductive fragmentation (Scheme 1), was studied. Under conditions that led to rapid increase in fluorescence for compound **37**, no fluorescence was observed for **36**.

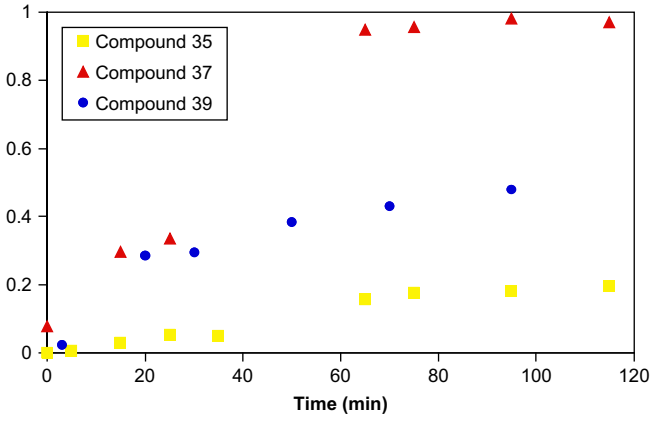


Figure 2. Increase in fluorescence upon release of 4-methyl-7-hydroxycoumarin **34** from compounds **35**, **37** and **39** following reduction with sodium dithionite.

A selection of the reductively activated prodrugs was assayed for their ability to inhibit VEGF-induced angiogenesis in human umbilical vein endothelial cells (HUVECs) in comparison to the free agents **15**–**17**. This assay examines the ability of human endothelial cells grown on a fibrin matrix to form three-dimensional structures when stimulated with the angiogenic promoter VEGF. The potency of each drug to inhibit this angiogenesis at a range of concentrations was then examined. HUVECs have been reported to express a range of bioreductive enzymes including NQO1,[49,50] and cytochrome c reductase (unpublished data from this laboratory) indicating that the cellular systems for bioreduction of the prodrugs are present.

Firstly, to control for the possible biological effects of compounds deriving from the fragmentation of the prodrug systems, such compounds were tested alone for their ability to inhibit VEGF-stimulated angiogenesis. Thus 4-aminobenzyl alcohol (a possible product from prodrugs **19**, **25** and **31**), oxindole (a possible product from prodrug **27**) and 3,3-dimethyloxindole (a possible product from prodrug **28**) were tested, but none of these compounds significantly inhibited angiogenesis at the concentrations tested. Secondly, to avoid the complication of hydrolytic cleavage of the ester bonds competing with the planned reductive fragmentation, the aryl esters **20**–**23** and **32** and **33** were omitted from this study. The results are summarized in Figure 3. In the biochanin A series, compound **18** that cannot be cleaved under bioreductive conditions is less potent than the free drug—it did not inhibit VEGF-stimulated angiogenesis to the same degree of significance as the free drug. The potential prodrug **19** appeared to exhibit a dose response, but is at least equipotent with free biochanin A. Although the ability of biochanin A to inhibit angiogenesis has also been noted elsewhere in the literature,[38] the molecular mechanism is not known.

The SU5416 (semaxanib) series of compounds are generally more potent than the isoflavones, with free SU5416 **16** itself causing 94% inhibition of VEGF-stimulated angiogenesis at 1 μM and 100% inhibition at 10 μM (Fig. 3). Again the benzyl derivative **24** that cannot undergo bioreductive fragmentation is considerably less potent than the free drug and does not significantly inhibit VEGF-induced angiogenesis at either 1 μM or 10 μM. However, its 4-nitrobenzyl analogue **25**, a potential prodrug, is more potent than **24**, inducing significant inhibition of VEGF-stimulated angiogenesis at 1 μM, suggesting that bioreductive release does occur. The similarity between the 3-and 2-nitrophenylacetyl compounds **26** and **27** suggests that the compounds may be undergoing hydrolytic rather than reductive fragmentation.

The 6-substituted derivatives of SU5416 **17** and **30**–**31** all appear equipotent (Fig. 3) with significant inhibition of VEGF-stimulated angiogenesis observed at 10 μM for all compounds, and with similar dose response profiles. A small number of 6-substituted derivatives of pyrrolylmethylidenyl oxindoles have been investigated previously; in the SU5416 series, the 6-fluoro-analogue is reported to be slightly more potent than its 6-unsubstituted counterpart,[40] whereas in the closely related SU6668 series of compounds that contain an additional 2-carboxyethyl group at the pyrrole 4-position, introduction of a large aryl substituent at the oxindole 6-position appears to increase potency.[41] Such an effect may be responsible for the activity of compounds **30** and **31**.

3. Conclusions

We have synthesized and evaluated a range of novel derivatives of the anti-angiogenic compounds biochanin A and SU5416 (semaxanib) as potential prodrug systems that can be bioreductively activated. Under chemical reducing conditions, the 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid based prodrugs appear to fragment the most efficiently, followed by the 2-nitrophenylacetate esters with the 4-nitrobenzyl ethers being the least efficient. In cellular systems all of the compounds that fragment rapidly by bioreduction significantly inhibited VEGF-stimulated angiogenesis at concentrations comparable to their parent compound, suggesting that the active drug is being released in a biological system. Several of the compounds, particularly the derivatives of SU5416 have potent anti-angiogenic activity, and form the basis of further study.

4. Experimental section

4.1. General procedures

Commercially available reagents were used throughout without purification unless otherwise stated. Light petroleum refers to the fraction with bp 40–60 °C and was distilled before use. Ether refers to diethyl ether. Reactions were routinely carried out under a nitrogen or argon atmosphere. Analytical thin layer chromatography was carried out on aluminium-backed plates coated with Merck Kieselgel 60 GF_{254}, and visualized under UV light at 254 and/or 360 nm. Chromatography was carried out using Merck Kieselgel 60H silica or Matrex silica 60. Fully characterized compounds were chromatographically homogeneous. Infrared spectra were recorded in the range 4000–600 cm^{-1} using Nicolet Magna FT-550 or Perkin Elmer FT-1600 spectrometers. NMR spectra were carried out on Bruker 300, 400 and 500 MHz instruments (^1H frequencies, corresponding ^{13}C frequencies are 75, 100 and 125 MHz). Chemical

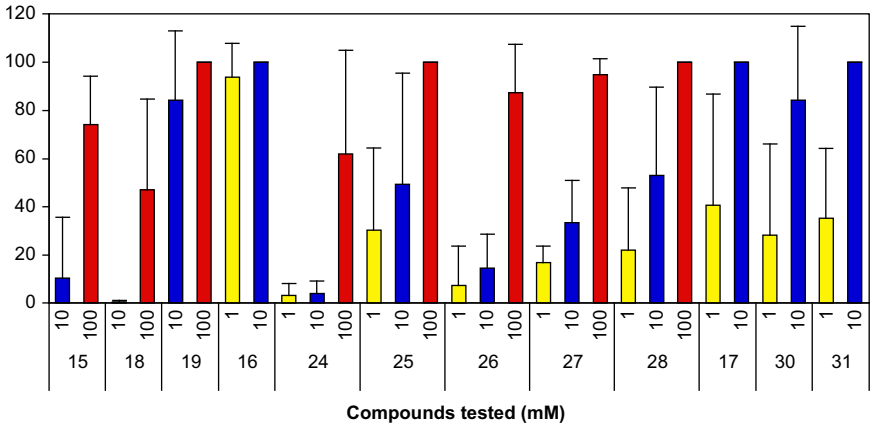


Figure 3. Inhibition of VEGF-stimulated angiogenesis in HUVECs by biochanin A **15**, SU5416 **16** and 6-hydroxy SU5416 **17** and various derivatives. HUVECs were seeded onto preformed fibrin matrices and treated with 100 ng/mL VEGF±test compounds at 1 (yellow), 10 (blue), or 100 (red) μM. After 5 days the formation of tubular structures was quantified. Results are expressed as percentage inhibition of a VEGF control and show mean±SD.

shifts are quoted in parts per million with TMS as internal standard. *J* values are recorded in hertz. In the ^{13}C spectra, signals corresponding to CH, CH_2 or CH_3 groups, as assigned from DEPT, are noted; all others are C. High and low resolution mass spectra were recorded on a Micromass GCT TDF High Resolution mass spectrometer, or at the EPSRC Mass Spectrometry Centre (Swansea).

4.1.1. 7-Benzyloxy 5-hydroxy-3-(4-methoxyphenyl)chromen-4-one ***18***

To biochanin A **15** (300 mg, 1.0 mmol) in DMF (9 mL) was added potassium carbonate (159 mg, 1.1 mmol) and the mixture was stirred at room temperature for 1.5 h. Benzyl bromide (0.14 mL, 1.1 mmol) was added and the mixture was stirred at room temperature for 4 h before adding water (5 mL). The product was extracted using ethyl acetate. The combined organic layers were washed with brine (2×10 mL) and were evaporated. The residue was purified by chromatography eluting with dichloromethane to give the *title compound* (200 mg, 51%) as a colourless solid; mp 197–199 °C (ethanol) (lit.,[51] mp 190 °C (acetone–methanol)). (Found: C, 74.0; H, 4.7. $C_{23}H_{18}O_5$ requires: C, 73.8; H, 4.8%.) ν_{max} (KBr)/cm^{-1} 3436, 1665, 1607, 1571, 1253, 1179, 1163; δ_H (300 MHz; $CDCl_3$) 12.87 (1H, br s, OH), 7.86 (1H, s, 2-H), 7.42 (7H, m, ArH), 6.98 (2H, dd, *J* 2.1, 6.8, ArH), 6.47 (2H, m, ArH), 5.13 (2H, s, CH_2), 3.85 (3H, s, OMe); δ_C (100 MHz; $CDCl_3$) 180.8 (C), 164.6 (C), 162.7 (C), 159.8 (C), 157.9 (C), 152.7 (CH), 135.7 (C), 130.1 (CH), 128.7 (CH), 128.4 (CH), 127.5 (CH), 123.7 (C), 122.9 (C), 114.1 (CH), 98.9 (CH), 93.3 (CH), 70.5 (CH_2), 55.4 (Me); one C unobserved; *m*/*z* (EI) 374 (M^+, 46%), 91 (100).

4.1.2. 5-Hydroxy-3-(4-methoxyphenyl)-7-(4-nitrobenzyloxy)-chromen-4-one ***19***

To a solution of biochanin A **15** (100 mg, 0.35 mmol) in DMF (3 mL) was added potassium hydride (21 mg, 0.52 mmol) portionwise and the mixture was stirred at room temperature for 1 h. 4-Nitrobenzyl bromide (91 mg, 0.42 mmol) was added and the solution was stirred at room temperature overnight. Water was added and the reaction mixture was extracted with ethyl acetate and toluene. The combined organic layers were dried ($MgSO_4$), filtered and evaporated in vacuo. Purification by chromatography eluting with ethyl acetate–toluene (1:4) gave the *title compound* (110 mg, 75%) as a colourless solid; mp 220–223 °C (ethanol). (Found: M^+, 419.1006. $C_{23}H_{17}NO_7$ requires: 419.1005.) ν_{max} (KBr)/cm^{-1} 3416, 3073, 2924, 2847, 1736, 1644, 1618; δ_H (400 MHz; $CDCl_3$) 12.90 (1H, s, OH), 8.28 (2H, d, *J* 8.8, ArH), 7.89 (1H, s, 2-H), 7.62 (2H, d, *J* 8.8, ArH), 7.46 (2H, dd, *J* 2.2, 6.7, ArH), 6.99 (2H, dd, *J* 2.2, 6.7, ArH), 6.47 (1H, d, *J* 2.3, ArH), 6.45 (1H, d, *J* 2.3, ArH), 5.24 (2H, s, CH_2), 3.85 (3H, s, OMe); δ_C (100 MHz; $CDCl_3$) 180.9 (C), 163.7 (C), 162.9 (C), 159.8 (C), 157.9 (C), 152.8 (C), 143.0 (C), 130.1 (CH), 127.7 (CH), 124.0 (CH), 123.8 (C), 122.7 (C), 114.1 (CH), 106.7 (C), 98.7 (CH), 93.3 (CH), 77.2 (CH), 68.9 (CH_2), 55.4 (Me); *m*/*z* (EI) 419 (M^+, 100), 284 (39), 255 (30), 132 (15), 105 (18), 78 (31).

4.1.3. 5-Hydroxy-3-(4-methoxyphenyl)-7-(3-nitrophenylacetoxy)-chromen-4-one ***20***

To biochanin A **15** (300 mg, 1.06 mmol), 3-nitrophenylacetic acid (147 mg, 0.81 mmol) and 4-dimethylaminopyridine (19 mg, 0.16 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 1.1 mL, 1.06 mmol) dropwise. The mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was purified by chromatography, eluting with dichloromethane to give the *title compound* (142 mg, 40%) as a colourless solid; mp 149–151 °C (ethanol). (Found: C, 64.4; H, 3.8; N, 3.1. $C_{24}H_{17}NO_8$ requires: C, 64.4; H, 3.8; N, 3.1%.) ν_{max} (KBr)/cm^{-1} 3467, 3080, 1763, 1608, 1529, 1515, 1350, 1251, 1144; δ_H (300 MHz; $CDCl_3$) 12.85 (1H, s, OH), 8.28 (1H, s, ArH), 8.21 (1H, d, *J* 8.2, ArH), 7.94 (1H, s, 2-H), 7.73 (1H, d, *J* 7.7, ArH), 7.58 (1H, m, ArH), 7.45 (2H, d, *J* 8.7, ArH), 6.98 (2H, d, *J* 8.7, ArH), 6.75 (1H, d, *J* 2.1, ArH), 6.56 (1H, d, *J* 2.1, ArH), 4.03 (2H, s, CH_2), 3.85 (3H, s, OMe); *m*/*z* (CI) 313 (18%), 285 (100), 164 (21).

4.1.4. 5-Hydroxy-3-(4-methoxyphenyl)-7-(2-nitrophenylacetoxy)-chromen-4-one ***21***

To a solution of biochanin A **15** (375 mg, 1.26 mmol) in toluene (15 mL) was added 2-nitrophenylacetic acid (456 mg, 2.52 mmol), triphenylphosphine (663 mg, 2.52 mmol) and diisopropyl azodicarboxylate (0.5 mL, 2.52 mmol) dropwise. The reaction mixture was stirred at room temperature for 18 h. Ethyl acetate was added and the organic phase was washed with sodium hydroxide solution (2 M), water and brine, dried ($MgSO_4$), filtered and concentrated. Purification by chromatography eluting with ethyl acetate–dichloromethane (1:19) gave the *title compound* (155 mg, 27%) as a colourless solid, mp 155–156 °C (ethanol). (Found: C, 64.4; H, 3.5; N, 2.9. $C_{24}H_{17}NO_8$ requires: C, 64.4; H, 3.8; N, 3.1%.) ν_{max} (KBr)/cm^{-1} 3431, 1772, 1649, 1608, 1577, 1511; δ_H (300 MHz; $CDCl_3$) 12.82 (1H, s, OH), 8.23 (1H, dd, *J* 8.2, 1.2, ArH), 7.95 (1H, s, 2-H), 7.68 (1H, m, ArH), 7.58 (1H, m, ArH), 7.49 (1H, d, *J* 6.7), 7.47 (2H, dd, *J* 2.1, 6.8, ArH), 7.00 (2H, dd, *J* 2.1, 6.8, ArH), 6.80 (1H, d, *J* 2.1, CH), 6.62 (1H, d, *J* 2.1, CH), 4.29 (2H, s, CH_2), 3.86 (3H, s, OMe); δ_C (75 MHz; $CDCl_3$) 181.7 (C), 168.1 (C), 162.8 (C), 160.3 (C), 157.3 (C), 156.2 (C), 153.7 (CH), 148.8 (C), 134.5 (CH), 134.0 (CH), 130.5 (CH), 129.6 (CH), 129.4 (C), 126.0 (CH), 124.5 (C), 122.9 (C), 114.5 (CH), 110.0 (C), 105.7 (CH), 101.3 (CH), 55.8 (Me), 40.6 (CH_2); *m*/*z* (CI) 404 (21%), 313 (51), 286 (26), 284 (100).

4.1.5. 5-Hydroxy-3-(4-methoxyphenyl)-7-[2-(2-nitrophenyl)-2,2-dimethylacetoxy]chromen-4-one ***22***

To biochanin A **15** (163 mg, 0.57 mmol), 2-methyl-2-(2-nitrophenyl)propanoic acid[7] (100 mg, 0.48 mmol) and 4-dimethylaminopyridine (11 mg, 0.01 mmol) in dichloromethane (4 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 0.57 mL, 0.57 mmol) dropwise. The mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by chromatography, eluting with dichloromethane to give the *title compound* (173 mg, 76%) as a pale yellow solid; mp 172–173 °C (ethanol). (Found: C, 65.8; H, 4.4; N, 3.0. $C_{26}H_{21}NO_8$ requires: C, 65.7; H, 4.5; N, 2.9%.) (Found: MH^+, 476.1343. $C_{26}H_{21}NO_8$+H requires: 476.1345.) ν_{max} (KBr)/cm^{-1} 3436, 1762, 1637, 1607, 1582, 1515, 1247, 1128; δ_H (300 MHz; $CDCl_3$) 12.83 (1H, s, OH), 8.05 (1H, d, *J* 7.7, ArH), 7.94 (1H, s, 2-H), 7.70 (2H, m, ArH), 7.51 (1H, m, ArH), 7.47 (2H, dd, *J* 1.9, 6.9, ArH), 6.99 (2H, dd, *J* 1.9, 6.9, ArH), 6.80 (1H, d, *J* 2.0, ArH), 6.54 (1H, d, *J* 2.0, ArH), 3.85 (3H, s, OMe), 1.84 (6H, s, Me); δ_C (100 MHz; $CDCl_3$) 181.3 (C), 172.9 (C), 162.3 (C), 159.9 (C), 156.9 (C), 156.1 (C), 153.3 (CH), 148.3 (C), 138.4 (C), 133.7 (CH), 130.1 (CH), 128.3 (CH), 128.1 (CH), 126.1 (CH), 124.0 (C), 122.6 (C), 114.1 (CH), 109.5 (C), 105.3 (CH), 101.1 (CH), 55.4 (Me), 46.6 (C), 27.2 (Me); *m*/*z* (CI) 476 (MH^+, 3%), 313 (18), 285 (100), 162 (14), 134 (18).

4.1.6. 5-Hydroxy-3-(4-methoxyphenyl)-7-[3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butan-1-oyloxy]chromen-4-one ***23***

To 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid (200 mg, 0.45 mmol), biochanin A **15** (356 mg, 1.35 mmol) and 4-dimethylaminopyridine (10 mg, 0.10 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 0.9 mL, 0.90 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue purified by chromatography, eluting with ethyl acetate–toluene (1:9) to give the *title compound* (197 mg, 87%) as a colourless solid; mp 110 °C (ethanol). (Found: MH^+, 503.1698. $C_{29}H_{26}O_8$+H requires: 503.1706.) ν_{max} (KBr)/cm^{-1} 3088, 2965, 2939, 1751, 1644, 1613, 1516, 1255, 1122; δ_H (300 MHz; $CDCl_3$) 12.80 (1H, s, OH), 7.92 (1H, s, 2-H), 7.44 (2H, dd, *J* 2.1, 6.7, ArH), 6.98 (2H, dd, *J* 2.1, 6.7, ArH), 6.63 (1H, d, *J* 2.1, ArH), 6.48 (1H, d, *J* 2.1, ArH), 6.46

(1H, s, ArH quinone), 3.84 (3H, s, OMe), 3.27 (2H, s, CH_2), 2.18 (3H, s, Me), 1.99 (3H, s, Me), 1.54 (6H, s, Me), irradiation of OH resulted in a 3.2% enhancement of H-6 signal and no enhancement of the H-8 signal; δ_C (75 MHz; $CDCl_3$) 191.0 (C), 187.1 (C), 181.2 (C), 170.3 (C), 162.5 (C), 160.0 (C), 156.9 (C), 155.6 (C), 155.6 (C), 153.2 (CH), 151.7 (C), 148.0 (C), 139.9 (C), 131.6 (CH), 130.1 (CH), 124.1 (C), 122.4 (C), 114.2 (CH), 109.5 (C), 105.3 (CH), 100.7 (CH), 55.3 (Me), 47.6 (CH_2), 38.7 (C), 15.9 (Me), 14.2 (Me), 28.9 (Me); *m*/*z* (CI) 313 (21%), 285 (100), 284 (13), 221 (14), 207 (10).

4.1.7. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]indolin-2-one (SU5416) **16**

Prepared in 77% yield from oxindole and 3,5-dimethylpyrrole-2-carboxaldehyde[45] as described in the literature;[40] mp 226–227 °C (ethanol) (lit.,[40] mp not given).

4.1.8. 1-Benzyl-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]indolin-2-one **24**

Prepared (93%) by benzylation of **16** as described in previously.[31]

4.1.9. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-1-(4-nitrobenzyl)indolin-2-one **25**

To a solution of SU5416 **15** (100 mg, 0.42 mmol) in DMF (3 mL) was added portionwise potassium hydride (washed from 30% in oil; 26 mg, 0.63 mmol) under a nitrogen atmosphere. The solution was stirred at room temperature for 1 h before adding 4-nitrobenzyl bromide (100 mg, 0.46 mmol) portionwise. The mixture was stirred at room temperature overnight and quenched with water. Brine was added to the reaction mixture, which was extracted with ether. The combined organic layers were dried ($MgSO_4$), filtered and evaporated in vacuo. The crude material was purified by chromatography, eluting with ethyl acetate–light petroleum (3:7) to give the *title compound* (97 mg, 62%) as a yellow orange solid; mp 195–198 °C (ethanol). (Found: MH^+, 374.1506. $C_{22}H_{19}N_3O_3$+H requires: 374.1504.) ν_{max} (KBr)/cm^{-1} 3047, 2919, 2852, 1675, 1567, 1347; δ_H (300 MHz; $CDCl_3$) 13.14 (1H, br s, NH), 8.18 (2H, d, *J* 8.8, ArH), 7.54 (1H, m, ArH), 7.47 (1H, s, =CH), 7.45 (2H, d, *J* 8.8, ArH), 7.10 (2H, m, ArH), 6.70 (1H, m, ArH), 6.01 (1H, d, *J* 2.5, pyrrole-H), 5.19 (2H, s, CH_2), 2.39 (3H, s, Me), 2.36 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 168.2 (C), 144.1 (C), 138.4 (C), 138.1 (C), 133.7 (C), 127.9 (CH), 127.2 (C), 125.7 (CH), 125.5 (C), 124.1 (CH), 123.8 (CH), 122.1 (CH), 117.3 (CH), 113.0 (CH), 110.7 (C), 108.2 (CH), 103.0 (C), 43.1 (CH_2), 14.0 (Me), 11.7 (Me).

4.1.10. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-1-(3-nitrophenylacetyl)indolin-2-one **26**

To SU5416 **16** (200 mg, 0.84 mmol), 3-nitrophenylacetic acid (228 mg, 1.26 mmol) and triphenylphosphine (331 mg, 1.26 mmol) in toluene (10 mL) was added diisopropyl azodicarboxylate (0.25 mL, 1.26 mmol) dropwise. The mixture was stirred at room temperature overnight, and then at 70 °C for 24 h. The solvent was evaporated and the residue was purified by chromatography, eluting with dichloromethane to give the *title compound* (19 mg, 6%) as an orange solid; mp 220–222 °C (ethanol). (Found: C, 68.55; H, 4.8; N, 10.2. $C_{23}H_{19}N_3O_4$ requires: C, 68.8; H, 4.8; N, 10.5%.) (Found: M^+, 401.1395. $C_{23}H_{19}N_3O_4$ requires: 401.1375.) ν_{max} (KBr)/cm^{-1} 3423, 1698, 1687, 1599, 1580, 1531, 1348, 1318, 1148; δ_H (300 MHz; $CDCl_3$) 12.60 (1H, br s, NH), 8.30 (1H, s, ArH), 8.23 (1H, m, ArH), 8.18 (1H, m, ArH), 7.72 (1H, m, ArH), 7.55 (1H, d, *J* 7.9, ArH), 7.51 (1H, m, ArH), 7.43 (1H, s, =CH), 7.21 (2H, m, ArH), 6.08 (1H, s, pyrrole-H), 4.70 (2H, s, CH_2), 2.46 (3H, s, Me), 2.36 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 171.0 (C), 168.6 (C), 148.3 (C), 138.9 (C), 136.4 (C), 136.2 (CH), 135.8 (C), 135.3 (C), 129.3 (CH), 127.5 (C), 126.7 (C), 126.1 (CH), 125.3 (CH), 124.7 (CH), 124.4 (CH), 122.2 (CH), 116.4 (CH), 116.3 (CH), 113.9 (CH), 109.3 (C), 44.3 (CH_2), 14.1 (Me), 11.8 (Me); *m*/*z* (EI) 401 (M^+, 50%), 238 (100), 221 (47), 209 (18), 136 (16), 89 (21).

4.1.11. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-1-(2-nitrophenylacetyl)indolin-2-one **27**

To a solution of SU5416 **16** (100 mg, 0.42 mmol) in toluene (5 mL) were added 2-nitrophenylacetic acid (152 mg, 0.84 mmol), triphenylphosphine (221 mg, 0.84 mmol) and diisopropyl azodicarboxylate (0.33 mL, 1.68 mmol) dropwise. The reaction mixture was stirred at room temperature for 18 h. Ethyl acetate was added and the organic phase was washed with sodium hydroxide solution (2 M), water and brine, dried ($MgSO_4$), filtered and concentrated in vacuo. Purification by chromatography eluting with ethyl acetate–light petroleum (1:4) gave the *title compound* (46 mg, 27%) as an orange solid; mp 203 °C (dichloromethane–ethanol). (Found: MH^+, 402.1476. $C_{23}H_{19}N_3O_4$+H requires: 402.1454.) ν_{max} (KBr)/cm^{-1} 3426, 3354, 2919, 1685, 1547, 1342; δ_H (300 MHz; $CDCl_3$) 12.58 (1H, br s, NH), 8.20 (1H, d, *J* 6.8, ArH), 8.16 (1H, d, *J* 9.4, ArH), 7.63 (1H, td, *J* 7.3, 1.0, ArH), 7.49 (3H, m, ArH), 7.42 (1H, s, =CH), 7.19 (2H, m, ArH), 6.06 (1H, s, pyrrole-H), 4.92 (2H, s, CH_2), 2.45 (3H, s, Me), 2.37 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 170.5 (C), 160.0 (C), 149.3 (C), 138.6 (C), 136.5 (C), 134.9 (C), 133.4 (CH), 133.1 (CH), 130.5 (C), 128.3 (CH), 127.4 (CH), 126.5 (C), 126.1 (CH), 125.1 (CH), 124.5 (CH), 124.2 (CH), 121.0 (C), 116.3 (CH), 113.6 (CH), 44.0 (CH_2), 14.0 (Me), 11.7 (Me); one C unobserved; *m*/*z* (CI) 402 (MH^+, 5%), 267 (17), 253 (10), 240 (18), 239 (100), 238 (48), 237 (10).

4.1.12. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-1-[2-(2-nitrophenyl)-2,2-dimethylacetyl]indolin-2-one **28**

To SU5416 **16** (300 mg, 1.26 mmol), 2-methyl-2-(2-nitrophenyl)propanoic acid[7] (272 mg, 1.30 mmol) and triphenylphosphine (397 mg, 1.51 mmol) in toluene (15 mL) was added diisopropyl azodicarboxylate (0.37 mL, 1.89 mmol) dropwise. The mixture was stirred at 70 °C overnight. The solvent was evaporated and the residue was purified twice by chromatography, eluting first with ethyl acetate–light petroleum (1:4) and then with dichloromethane–light petroleum (1:1) to give the *title compound* (27 mg, 5%) as an orange solid; mp 147–150 °C (ethanol). (Found: C, 69.8; H, 5.6; N, 9.7. $C_{25}H_{23}N_3O_4$ requires: C, 69.9; H, 5.4; N, 9.8%.) (Found: M^+, 429.1674. $C_{25}H_{23}N_3O_4$ requires: 429.1688.) ν_{max} (KBr)/cm^{-1} 3436, 2927, 1700, 1686, 1565, 1523, 1343, 1289, 1260, 1148; δ_H (300 MHz; $CDCl_3$) 12.02 (1H, br s, NH), 8.10 (1H, d, *J* 9.2, ArH), 7.68 (2H, m, ArH), 7.54 (1H, m, ArH), 7.42 (1H, dd, *J* 1.3, 7.6, ArH), 7.25 (2H, m, ArH), 7.22 (1H, s, =CH), 7.17 (1H, dt, *J* 1.2, 7.6, ArH), 5.93 (1H, s, pyrrole-H), 2.28 (3H, s, Me), 2.25 (3H, s, Me), 1.93 (6H, s, Me); δ_C (100 MHz; $CDCl_3$) 176.5 (C), 166.5 (C), 148.2 (C), 139.7 (C), 137.2 (C), 136.1 (C), 133.8 (C), 132.0 (CH), 129.3 (CH), 127.0 (C), 126.9 (C), 126.8 (CH), 126.2 (CH), 125.0 (CH), 124.2 (CH), 123.5 (CH), 116.3 (CH), 116.1 (CH), 113.0 (CH), 109.8 (C), 50.4 (C), 27.6 (Me), 13.8 (Me), 11.6 (Me); *m*/*z* (EI) 429 (M^+, 18), 237 (100), 209 (42).

4.1.13. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-1-[3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butan-1-oyl]indolin-2-one **29**

To 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid[44] (245 mg, 1.04 mmol), SU5416 **16** (295 mg, 1.24 mmol) and 4-dimethylaminopyridine (24 mg, 0.02 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 1.24 mL, 1.24 mmol) dropwise. The mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by chromatography, eluting with dichloromethane to give the *title compound* (215 mg, 52%) as an orange solid; mp 125–127 °C (ethanol). (Found: M^+, 456.2029. $C_{28}H_{28}N_2O_4$ requires: 456.2049.) ν_{max} (KBr)/cm^{-1} 3423, 2924, 1638, 1650, 1636, 1540, 1355, 1315, 1262, 1149, 1081; δ_H (300 MHz; $CDCl_3$) 12.54 (1H, br s, NH), 8.11 (1H, m, ArH), 7.50 (1H, m, ArH), 7.41 (1H, s, =CH), 7.18 (2H, m, ArH), 6.49 (1H, d, *J* 1.5, CH), 6.07 (1H, d, *J* 2.3, pyrrole-H), 3.97 (2H, s, CH_2), 2.47 (3H, s, Me), 2.37 (3H, s, Me), 2.21 (3H, s, Me), 1.93 (3H, s, Me), 1.58 (6H, s, Me); δ_C (100 MHz; $CDCl_3$) 191.3 (C), 187.6 (C), 174.0 (C), 168.5 (C), 154.5 (C), 148.3 (C), 138.3 (C), 137.5 (C),

136.0 (C), 134.6 (C), 131.4 (CH), 127.3 (C), 126.6 (C), 125.9 (CH), 124.3 (CH), 124.0 (CH), 116.3 (CH), 116.0 (CH), 113.6 (CH), 109.9 (C), 52.8 (CH_2), 37.9 (C), 28.7 (Me), 15.9 (Me), 14.2 (Me), 14.1 (Me), 11.7 (Me); *m*/*z* (CI) 457 (MH^+, 43%), 456 (M^+, 21), 240 (13), 239 (100), 238 (77), 221 (65), 220 (10).

4.1.14. 6-Hydroxy-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]-indolin-2-one **17**

A solution of 6-hydroxyoxindole[46] (150 mg, 1.01 mmol), pyrrolidine (0.13 mL, 1.51 mmol) and 3,5-dimethylpyrrole-2-carboxaldehyde[45] (149 mg, 1.21 mmol) in ethanol (10 mL) was heated to reflux at 90 °C for 4 h. The ethanol was removed in vacuo and the crude product was purified by chromatography eluting with ethyl acetate–light petroleum (2:3) to give the *title compound* (225 mg, 88%) as an orange crystalline solid; mp >300 °C (from ethyl acetate–light petroleum). (Found: M^+, 254.1055. $C_{15}H_{14}N_2O_2$ requires: 254.1055.) ν_{max} (KBr)/cm^{-1} 3697, 3606, 1602, 1266, 1105; δ_H (400 MHz; DMSO-*d*) 13.07 (1H, s, NH), 10.57 (1H, s, OH), 9.35 (1H, s, NH), 7.44 (1H, d, *J* 8.2, ArH), 7.28 (1H, s, =CH), 6.36 (1H, dd, *J* 8.2, 2.2, ArH), 6.31 (1H, d, *J* 2.2, ArH), 5.91 (1H, d, *J* 2.1, pyrrole-H), 2.26 (3H, s, Me), 2.23 (3H, s, Me); δ_C (100 MHz; DMSO-*d*) 170.4 (C), 157.1 (C), 140.4 (C), 134.2 (C), 129.8 (C), 126.8 (C), 121.1 (CH), 119.7 (CH), 117.5 (C), 114.2 (C), 112.3 (CH), 108.6 (CH), 97.7 (CH), 13.9 (Me), 11.7 (Me); *m*/*z* (EI) 254 (M^+, 100%).

4.1.15. 6-Benzyloxy-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]-indolin-2-one **30**

(a) To 6-hydroxyoxindole[46] (50 mg, 0.336 mmol) in methanol (5 mL) was added potassium carbonate (48.6 mg, 0.35 mmol) and benzyl bromide (42 μL, 0.35 mmol). The solution was heated to reflux for 2.5 h. On cooling the reaction mixture was diluted with ethyl acetate (20 mL) and washed with sodium hydroxide (2 M; 10 mL) and water (10 mL). The solvent was removed in vacuo and the crude product was purified by chromatography eluting with ethyl acetate–light petroleum (1:4 to 2:3) to give 6-benzyloxyoxindole (40 mg, 50%) as an pale pink sticky solid used without further purification; δ_H (300 MHz; $CDCl_3$) 7.67 (1H, s, NH), 7.46–7.34 (5H, m, ArH), 7.13 (1H, d, *J* 8.1, ArH), 6.64 (1H, dd, *J* 8.1, 2.1, ArH), 6.55 (1H, d, *J* 2.1, ArH), 5.10 (2H, s, CH_2), 3.49 (2H, s, CH_2); δ_C (100 MHz; $CDCl_3$) 179.4 (C), 159.5 (C), 144.1 (C), 137.2 (C), 128.9 (CH), 128.3 (CH), 127.8 (CH), 125.5 (CH), 117.8 (C), 108.7 (C), 98.4 (CH), 70.6 (CH_2), 36.2 (CH_2).

(b) A solution of 6-benzyloxyoxindole (35 mg, 0.15 mmol), pyrrolidine (0.03 mL, 0.35 mmol) and 3,5-dimethylpyrrole-2-carboxaldehyde[45] (34.7, 0.28 mmol) in ethanol (4 mL) was heated to reflux at 90 °C for 4 h. The ethanol was removed in vacuo and the crude product was purified by chromatography eluting with ethyl acetate–light petroleum (2:3) to give the *title compound* (45 mg, 89%) as an orange crystalline solid; mp >241–243 °C (from ethyl acetate–light petroleum). (Found: M^+, 344.1519. $C_{22}H_{20}N_2O_2$ requires: 344.1525.) ν_{max} (KBr)/cm^{-1} 3697, 3450, 1674, 1631, 1597, 1570, 1455, 1366, 1312, 1279; δ_H (400 MHz; $CDCl_3$) 12.90 (1H, br s, NH), 7.62 (1H, br s, NH), 7.45–7.25 (7H, m, 6×ArH and =CH), 6.69 (1H, dd, *J* 8.4, 2.3, ArH), 6.55 (1H, d, *J* 2.3, ArH), 5.95 (1H, d, *J* 2.6, pyrrole-H), 5.10 (2H, s, CH_2), 2.36 (3H, s, Me), 2.31 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 170.1 (C), 157.9 (C), 138.0 (C), 137.0 (C), 135.9 (C), 131.4 (C), 128.6 (CH), 128.0 (CH), 127.4 (CH), 127.0 (C), 122.1 (CH), 119.8 (C), 118.3 (CH), 112.3 (CH), 111.9 (C), 108.2 (CH), 97.2 (CH), 70.4 (CH_2), 13.9 (Me), 11.6 (Me); *m*/*z* (ES) 344 (M^+, 3%), 253 (100%), 91 (2%).

4.1.16. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-6-(4-nitrobenzyloxy)indolin-2-one **31**

To 6-hydroxy-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]indolin-2-one **17** (30 mg, 0.118 mmol) in DMF (2 mL) was added potassium carbonate (17 mg, 0.12 mmol). The reaction mixture was stirred for 10 min and then 4-nitrobenzyl bromide (27 mg, 0.12 mmol) was added and the reaction mixture stirred at rt for 3 h. The solution was then heated to 60 °C for 1 h. On cooling the reaction mixture was diluted with ethyl acetate (20 mL) and washed with water (2×20 mL) and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was recrystallized from ethyl acetate–methanol to give the *title compound* (27.5 mg, 60%) as an yellow crystalline solid; mp 253–255 °C (decomp.) (from ethyl acetate–methanol). (Found: MH^+, 390.1438. $C_{22}H_{19}N_3O_4$+H requires: 390.1454.) ν_{max} (KBr)/cm^{-1} 3449, 1674, 1631, 1568, 1348, 1312, 1150, 1102; δ_H (500 MHz; DMSO-*d*) 13.15 (1H, br s, NH), 10.76 (1H, s, NH), 8.27 (2H, d, *J* 8.5, ArH), 7.73 (2H, d, *J* 8.5, ArH), 7.63 (1H, d, *J* 8.5, ArH), 7.42 (1H, s, =CH), 6.67 (1H, dd, *J* 8.5, 2.5, ArH), 6.53 (1H, d, *J* 2.5, ArH), 5.96 (1H, d, *J* 2.0, pyrrole-H), 5.29 (2H, s, CH_2), 2.32 (3H, s, Me), 2.27 (3H, s, Me); δ_C (100 MHz; DMSO-*d*) 169.8 (C), 157.0 (C), 146.9 (C), 145.2 (C), 139.3 (C), 134.6 (C), 130.4 (C), 128.1 (CH), 126.4 (C), 123.5 (CH), 121.8 (CH), 119.2 (C), 119.1 (CH), 112.8 (CH), 112.1 (C), 107.6 (CH), 96.8 (CH), 68.2 (CH_2), 13.4 (Me), 11.2 (Me); *m*/*z* (ES) 390 (MH^+, 100%), 263 (95), 149 (80).

4.1.17. 3-[(3,5-Dimethylpyrrol-2-yl)methylidenyl]-6-(2-nitrophenylacetoxy)indolin-2-one **32**

To 6-hydroxy-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]indolin-2-one **17** (30.0 mg, 0.118 mmol), 2-nitrophenylacetic acid (21.4 mg, 0.128 mmol) and 4-dimethylaminopyridine (2.5 mg, 24 μmol) in dichloromethane (2 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 0.118 mL) dropwise. The mixture was stirred at room temperature for 18 h. Dichloromethane was added (10 mL) and the organic layer was washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and solvent removed in vacuo. The crude product was purified by chromatography eluting with dichloromethane–ethyl acetate (9:1) and recrystallization from ethanol and then ethyl acetate–hexane to give the *title compound* (11 mg, 22%) as a yellow crystalline solid; mp 201–203 °C (from ethyl acetate–hexane). (Found: MH^+, 418.1413. $C_{23}H_{19}N_3O_5$+H requires: 418.1403.) ν_{max} (KBr)/cm^{-1} 3697, 1674, 1601, 1568, 1349, 1140; δ_H (500 MHz; $CDCl_3$) 13.02 (1H, br s, NH), 8.20 (1H, d, *J* 8.0, ArH), 7.67–7.64 (2H, m, NH and ArH), 7.53 (1H, t, *J* 8.0, ArH), 7.46 (1H, d, *J* 8.0, ArH), 7.41 (1H, d, *J* 8.5, ArH), 7.32 (1H, s, =CH), 6.78 (1H, dd, *J* 8.5, 2.0, ArH), 6.70 (1H, d, *J* 2.0, ArH), 5.97 (1H, d, *J* 0.5, pyrrole-H), 4.26 (2H, s, CH_2), 2.37 (3H, s, Me), 2.31 (3H, s, Me); δ_C (100 MHz; DMSO-*d*) 169.5 (C), 169.1 (C), 148.5 (C), 148.1 (C), 138.4 (C), 135.9 (C), 134.2 (CH), 133.9 (CH), 131.9 (C), 129.6 (C), 129.1 (CH), 126.6 (C), 125.0 (CH), 123.6 (CH), 123.2 (C), 118.6 (CH), 113.6 (CH), 112.6 (CH), 111.7 (C), 103.2 (CH), 40.0 (CH_2), 13.5 (Me), 11.2 (Me); *m*/*z* (ES) 418 (MH^+, 100%), 371 (17), 223 (17).

4.1.18. 6-[3-Methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoyloxy]-3-[(3,5-dimethylpyrrol-2-yl)methylene]-indolin-2-one **33**

To 6-hydroxy-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]indolin-2-one **17** (32 mg, 0.126 mmol), 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid[44] (29.7 mg, 0.126 mmol), 4-dimethylaminopyridine (2.5 mg, 24 μmol) and triethylamine (17.6 μL, 0.126 mmol) in dichloromethane (3 mL) was added 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (24.2 mg, 0.126 mmol) portionwise. The mixture was stirred at room temperature for 18 h. Dichloromethane was added (10 mL) and the organic layer was washed with water (10 mL), brine (10 mL), dried ($MgSO_4$) and solvent removed in vacuo. The crude product was purified by chromatography eluting with ethyl acetate–light petroleum (1:1) and recrystallized from ethyl acetate–hexane to give the *title compound* (25 mg, 42%) as an yellow crystalline solid; mp 177–179 °C (from ethyl acetate–hexane). (Found: MH^+, 473.2074. $C_{28}H_{28}N_2O_5$+H requires: 473.2077.) ν_{max} (KBr)/cm^{-1} 3449, 2986, 1710, 1673, 1568, 1363, 1133; δ_H (500 MHz; $CDCl_3$) 13.00 (1H, br s, NH), 7.74 (1H, s, NH), 7.39 (1H, d, *J* 8.5, ArH), 7.32 (1H, s, ArH), 6.64

(1H, dd, *J* 8.5, 2.0, ArH), 6.56 (1H, d, *J* 2.0, ArH), 6.43 (1H, d, *J* 1.5, ArH), 5.98 (1H, br s, pyrrole-H), 3.24 (2H, s, CH_2), 2.37 (3H, s, Me), 2.31 (3H, s, Me), 2.17 (3H, s, Me), 1.97 (3H, d, *J* 1.5, Me), 1.54 (6H, s, Me); δ_C (100 MHz; $CDCl_3$) 191.1 (C), 187.2 (C), 171.6 (C), 169.8 (C), 152.3 (C), 148.4 (C), 148.2 (C), 139.5 (CH), 137.3 (C), 137.2 (C), 133.1 (C), 131.5 (CH), 127.2 (C), 124.4 (C), 123.7 (CH), 117.8 (CH), 114.4 (CH), 112.9 (CH), 110.7 (C), 103.3 (CH), 47.6 (CH_2), 38.8 (C), 29.0 (Me), 16.0 (Me), 14.2 (Me), 14.0 (Me), 11.7 (Me); *m*/*z* (ES) 473 (MH^+, 100%), 413 (22), 371 (20), 363 (22), 358 (20), 282 (24), 223 (23).

4.1.19. 4-Methyl-7-(4-nitrobenzyloxy)coumarin **35**

To 4-methyl-7-hydroxycoumarin **34** (400 mg, 2.2 mmol) in DMF (20 mL) was added potassium hydride (136 mg, 3.4 mmol) portionwise. The mixture was stirred at room temperature for 1 h before adding 4-nitrobenzyl bromide (732 mg, 3.4 mmol). The mixture was then stirred at room temperature overnight. Water was then added and the mixture was extracted with ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated in vacuo. The solid obtained was stirred in light petroleum, filtered and rinsed with light petroleum and a small amount of ethyl acetate to give the *title compound* (670 mg, 95%) as a colourless solid; mp 203–204 °C (ethanol). (Found: MH^+, 312.0880. $C_{17}H_{13}NO_5$+H requires: 312.0872.) ν_{max} (KBr)/cm^{-1} 3067, 1721, 1623, 1603, 1516, 1388, 1342, 1285; δ_H (300 MHz; $CDCl_3$) 8.27 (2H, d, *J* 8.7, ArH), 7.63 (2H, d, *J* 8.7, ArH), 7.54 (1H, d, *J* 8.8, ArH), 6.95 (1H, dd, *J* 2.5, 8.8, ArH), 6.87 (1H, d, *J* 2.5, ArH), 6.18 (1H, s, 3-H), 5.34 (2H, s, CH_2), 2.41 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 161.4 (C), 161.2 (C), 155.5 (C), 152.8 (C), 148.0 (C), 143.5 (C), 128.1 (CH), 126.2 (CH), 124.4 (CH), 114.6 (C), 113.1 (CH), 112.9 (CH), 102.3 (CH), 69.4 (CH_2), 19.1 (Me); *m*/*z* (CI) 312 (MH^+, 100%), 177 (81), 106 (18).

4.1.20. 4-Methyl-7-(3-nitrophenylacetoxy)coumarin **36**

To 3-nitrophenylacetic acid (188 mg, 1.04 mmol), 4-methyl-7-hydroxycoumarin **34** (219 mg, 1.24 mmol) and 4-dimethylaminopyridine (24.3 mg, 0.025 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 1.24 mL, 1.24 mmol) dropwise. The mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by chromatography, eluting with dichloromethane to give the *title compound* (255 mg, 72%) as a colourless solid; mp 188–190 °C (ethanol). (Found: C, 63.7; H, 3.9; N, 4.1. $C_{18}H_{13}NO_6$ requires: C, 63.7; H, 3.9; N, 4.1%.) (Found: MH^+, 340.0831. $C_{18}H_{13}NO_6$+H requires: 340.0821.) ν_{max} (KBr)/cm^{-1} 3093, 3069, 2927, 1751, 1615, 1533, 1522, 1388, 1351, 1262, 1135; δ_H (300 MHz; $CDCl_3$) 8.29 (1H, s, ArH), 8.23 (1H, d, *J* 8.2, ArH), 7.75 (1H, d, *J* 7.7, ArH), 7.61 (2H, m, ArH), 7.13 (1H, d, *J* 2.3, ArH), 7.08 (1H, dd, *J* 2.3, 8.6, ArH), 6.29 (1H, s, 3-H), 4.05 (2H, s, CH_2), 2.45 (3H, s, Me); δ_C (100 MHz; $CDCl_3$) 168.3 (C), 160.4 (C), 154.1 (C), 152.7 (C), 151.8 (C), 135.6 (CH), 134.6 (C), 129.8 (CH), 125.5 (CH), 124.5 (CH), 122.8 (CH), 118.1 (C), 117.8 (CH), 114.8 (CH), 110.3 (CH), 40.6 (CH_2), 18.8 (Me); one C unobserved; *m*/*z* (CI) 340 (MH^+, 15%), 177 (100), 164 (18).

4.1.21. 4-Methyl-7-(2-nitrophenylacetoxy)coumarin **37**

To 4-methyl-7-hydroxycoumarin **34** (400 mg, 2.2 mmol) in toluene (24 mL) was added 2-nitrophenylacetic acid (614 mg, 3.4 mmol), triphenylphosphine (460 mg, 3.4 mmol) and diisopropyl azodicarboxylate (0.66 mL, 3.4 mmol) dropwise. The solution was stirred at room temperature for 48 h. The mixture was then filtered and the solid was rinsed with light petroleum and a small amount of ethyl acetate to give the *title compound* (248 mg, 32%) as a colourless solid; mp 157–158 °C (ethanol). (Found: C, 63.9; H, 3.7; N, 3.9. $C_{18}H_{13}NO_6$ requires: C, 63.7; H, 3.9; N, 4.1%.) (Found: MH^+, 340.0826. $C_{18}H_{13}NO_6$+H requires: 340.0821.) ν_{max} (KBr)/cm^{-1} 3078, 1762, 1731, 1710, 1603, 1513; δ_H (300 MHz; $CDCl_3$) 8.22 (1H, dd, *J* 1.1, 8.1, ArH), 7.66 (1H, m, ArH), 7.57 (2H, m, ArH), 7.48 (1H, dd, *J* 1.0, 7.5, ArH), 7.13 (2H, dd, *J* 2.2, 6.5, ArH), 6.28 (1H, d, *J* 1.0, 3-H), 4.00 (2H, s, CH_2), 2.44 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 168.5 (C), 160.9 (C), 154.5 (C), 153.4 (C), 152.3 (C), 134.5 (CH), 134.0 (CH), 129.6 (CH), 129.4 (C), 126.0 (CH), 125.8 (CH), 118.5 (CH), 118.4 (C), 115.0 (CH), 110.8 (CH), 90.5 (C), 40.6 (CH_2), 19.6 (Me); *m*/*z* (CI) 340 (MH^+, 9%), 177 (100).

4.1.22. 4-Methyl-7-[2,2-dimethyl-2-(2-nitrophenyl)acetoxy]-coumarin **38**

To 2-methyl-2-(2-nitrophenyl)propanoic acid[7] (217 mg, 1.04 mmol), 4-methyl-7-hydroxycoumarin **34** (219 mg, 1.24 mmol) and 4-dimethylaminopyridine (24 mg, 0.02 mmol) in dichloromethane (5 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 1.24 mL, 1.24 mmol) dropwise. The mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by chromatography, eluting with ethyl acetate–light petroleum (1:1) to give the *title compound* (234 mg, 61%) as a colourless solid; mp 139–140 °C (ethanol). (Found: C, 65.0; H, 4.4; N, 3.7. $C_{20}H_{17}NO_6$ requires: C, 65.4; H, 4.7; N, 3.8%.) (Found: MH^+, 368.1128. $C_{20}H_{17}NO_6$+H requires: 368.1134.) ν_{max} (KBr)/cm^{-1} 1750, 1728, 1523, 1353; δ_H (300 MHz; $CDCl_3$) 8.05 (1H, d, *J* 7.6, ArH), 7.71 (2H, m, ArH), 7.60 (1H, d, *J* 8.8, ArH), 7.52 (1H, m, ArH), 7.13 (1H, dd, *J* 2.2, 8.6, ArH), 7.07 (1H, m, ArH), 6.27 (1H, s, 3-H), 2.43 (3H, s, Me), 1.85 (6H, s, Me); δ_C (75 MHz; $CDCl_3$) 173.8 (C), 160.6 (C), 154.1 (C), 153.2 (C), 152.0 (C), 148.3 (C), 138.5 (C), 133.9 (CH), 128.3 (CH), 128.1 (CH), 126.1 (CH), 125.4 (CH), 118.3 (CH), 118.0 (C), 114.6 (CH), 110.4 (CH), 46.6 (C), 27.3 (Me), 18.8 (Me); *m*/*z* (CI) 368 (MH^+, 100%), 192 (45), 177 (100), 164 (24), 148 (17), 134 (21).

4.1.23. 4-Methyl-7-[3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butan-1-oyloxy]coumarin **39**

To 3-methyl-3-(3,6-dimethyl-1,4-benzoquinon-2-yl)butanoic acid (250 mg, 1.1 mmol), 4-methyl-7-hydroxycoumarin **34** (400 mg, 2.2 mmol) and dimethylaminopyridine (13 mg, 0.1 mmol) in dichloromethane (6 mL) was added dicyclohexylcarbodiimide (1 M in dichloromethane; 1.1 mL, 1.1 mmol) dropwise and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue was purified by chromatography eluting with ethyl acetate–light petroleum (2:3) to give the *title compound* (151 mg, 34%) as a colourless solid; mp 117 °C (ethanol). (Found: MH^+, 395.1477. $C_{23}H_{22}O_6$+H requires: 395.1494.) ν_{max} (KBr)/cm^{-1} 3431, 2950, 2914, 1751, 1726, 1654, 1613, 1127, 1106; δ_H (300 MHz; $CDCl_3$) 7.58 (1H, d, *J* 8.5, ArH), 7.02 (1H, d, *J* 2.2, ArH), 6.96 (1H, dd, *J* 2.2, 8.5, ArH), 6.46 (1H, d, *J* 1.5, ArH quinone), 6.27 (1H, d, *J* 1.1, ArH), 3.29 (2H, s, CH_2), 2.42 (3H, d, *J* 1.3, Me), 2.18 (3H, s, Me), 1.99 (3H, d, *J* 1.5, Me), 1.55 (6H, s, Me); δ_C (75 MHz; $CDCl_3$) 214.8 (C), 207.1 (C), 191.4 (C), 187.5 (C), 171.2 (C), 157.7 (C), 153.1 (C), 152.2 (C), 148.0 (C), 140.2 (C), 132.0 (CH), 125.8 (CH), 118.5 (C), 118.3 (CH), 115.0 (CH), 110.8 (CH), 47.9 (CH_2), 29.3 (Me), 19.1 (Me), 16.4 (Me), 14.6 (Me); one C unobserved; *m*/*z* (CI) 395 (MH^+, 8%), 221 (48), 177 (100), 148 (5).

4.1.24. Reduction of 3-[(3,5-dimethylpyrrol-2-yl)methylene]-1-(4-nitrobenzyl)indolinone **25**

To 3-[(3,5-dimethylpyrrol-2-yl)methylene]-1-(4-nitrobenzyl)-indolinone **25** (18 mg, 0.05 mmol) in ethanol (0.5 mL) was added ammonium chloride saturated solution (0.1 mL) and indium powder (40 mg, 0.35 mmol). The mixture was heated under reflux for 4 h and then cooled to room temperature, water (1 mL) was added and the pH was adjusted to 9 with sodium hydroxide solution (2 M). The product was extracted with ethyl acetate, dried ($MgSO_4$), filtered and the solvent evaporated in vacuo. Purification by chromatography eluting with ethyl acetate–light petroleum (3:7) gave *1-(4-aminobenzyl)-3-[(3,5-dimethylpyrrol-2-yl)methylidenyl]indolin-2-one* (16 mg, 90%) as a yellow solid; mp 212–215 °C (ethanol). (Found: MH^+, 344.1776. $C_{22}H_{21}N_3O$+H requires: 344.1763.) ν_{max}

(KBr)/cm^{-1} 3447, 3360, 2924, 2847, 1659, 1567, 1460, 1347, 1265, 1178; δ_H (300 MHz; $CDCl_3$) 13.28 (1H, br s, NH), 7.60 (1H, d, *J* 7.3, ArH), 7.43 (1H, s, =CH), 7.13 (2H, d, *J* 8.1, ArH), 7.06 (2H, m, ArH), 6.81 (1H, d, *J* 7.3, ArH), 6.63 (2H, d, *J* 8.3, ArH), 5.98 (1H, s, pyrrole-H), 4.98 (2H, s, CH_2), 3.57 (2H, br s, NH_2), 2.39 (3H, s, Me), 2.34 (3H, s, Me); δ_C (75 MHz; $CDCl_3$) 165.5 (C), 146.0 (C), 139.4 (C), 137.0 (C), 132.6 (C), 128.8 (CH), 127.4 (C), 126.8 (C), 126.0 (C), 125.9 (CH), 123.5 (CH), 121.9 (CH), 117.4 (CH), 115.7 (CH), 112.9 (CH), 112.2 (C), 109.2 (CH), 30.1 (CH_2), 14.3 (Me), 12.0 (Me); *m*/*z* (CI) 344 (MH^+, 85%), 343 (M^+, 100), 251 (96).

4.1.25. *In vitro angiogenesis model*

Angiogenesis assays were carried out using a method based on that of Koolwijk et al.[52] Human fibrinogen (5 mg/mL) was dissolved in serum free Hams F12 and filtered through a 0.2 μm filter. Fibrin matrices were prepared by polymerizing the solution of fibrinogen with 1.5 U/mL of human thrombin. Six hundred microlitre of this mixture was added to each well of a 24 well plate. After polymerization, matrices were soaked in Hams F12 containing 20% FCS for 120 min at 37 °C to inactivate the thrombin. Highly confluent HUVECs were plated onto the surface of the matrix and cultured for 24 h. After this time the medium was replaced with medium containing anti-angiogenic agents ±100 ng/mL of VEGF. Drugs were tested at concentrations shown in Figure 3. Controls received VEGF and carrier alone. After a maximum of 5 days the formation of tubular structures of endothelial cells in the three-dimensional matrix was analyzed. Two randomly chosen microscopic fields per well were examined and the number of times a tubular structure crossed a 1 cm grid line were scored to provide an index of the degree of angiogenesis in each well. Each condition was carried out in triplicate wells, and each drug was tested using 2–4 different cell populations. Since assays were performed on primary cells isolated from different individuals a certain degree of variability was observed in cell responses and this was reflected in the standard deviations observed. Statistical analyses were carried out on the raw data using the using the Mann–Whitney U Test and $P<0.05$ was considered statistically significant. Values are expressed as mean±SD.

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